stating that the content of the "Sequence Listing" in the paper form and computer readable form are the same, and, a statement under 37 C.F.R. §1.821(g), stating that no new matter is included in the application.

Applicant respectfully submits that a sequence listing statement under 37 C.F.R. §1.821 (f) was submitted with the filing of the instant application on 12/01/94. Confirmation of receipt was acknowledged by the USPTO on 12/01/94 by stamping and returning the confirmation postcard to Applicant. Copies of the Application Fee Transmittal form with the sequence listing statement and confirmation postcard are enclosed herewith. Therefore, Applicant believes that a statement under 37 C.F.R. §1.821(g) is not necessary, and requests that the objection be withdrawn.

Claims 9-30 have been rejected under 35 U.S.C. §112, first paragraph, for failing to provide an enabling disclosure. It is the belief of the Examiner that the disclosure enables only a method for stimulating erythropoiesis with thrombopoietin, as shown in SEQ ID NO:2, with and without erythropoietin.

Specifically, the Examiner alleges that claims 9-13, 15-22 and 25-30 encompass "TPO" from any species, which could include thyroid peroxidase or thymopoietin, and therefore are too broad. Regarding the rejection of claims 15 and 25, the Examiner states that the claims read on fragments of SEQ ID NO: 2. With respect to claims 28-30, the Examiner alleges that the claims recite: "..in an amount sufficient for increasing reticulocyte counts at least 2-fold over baseline reticulocyte counts" and are not enabled. The Examiner states that it appears that Figure 2 does not demonstrate that reticulocyte counts returned to 2-fold over baseline when animals are treated with thrombopoietin.

Applicant respectfully traverses the rejection. However, Applicant has amended claims 9, 18, 28, 29, and 30 to recite a thrombopoietin protein selected from the group

consisting of: (a) proteins comprising the sequence of amino acids of SEQ ID NO: 4, from amino acid residue 45 to amino acid residue 379; allelic variants of (a); and species homologs of (a) or (b). All other claims have been amended to recite thrombopoietin instead of TPO or incorporate such amendments by virtue of their dependency on amended claims. These amendments have been made merely for purposes of clarification and thus, Applicant believes this aspect of the rejection is obviated.

With regard to the Examiner's contention that claims 15 and 25 read on fragments of thrombopoietin, but such fragments are not enabled, Applicant has amended claims 15 and 25 to recite certain preferred embodiments. The amended claims recite: "comprises a sequence of amino acids selected from the group consisting of the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 1 to residue 353 and the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 22 to residue 353." While Applicant does not agree that claim 15 and claim 25, which recite specific fragments that (by virtue of their dependency on claims 9 and 18) have the ability to "produce an increase in proliferation or differentiation of erythroid cells," are not enabled, the Examiner suggests that there are different issues of patentability for these claims and Applicant prefers to address these issues independently. Applicant submits that cancellation of the recitation of specific fragments is made to expedite prosecution and allowance of the remaining claims, and reserves the right to prosecute the omitted subject matter in one or more continuing applications.

Regarding the Examiner's rejection of claims 28-30, Applicant has amended the claims to recite: "..in an amount two-fold over baseline reticulocyte counts within fourteen days." The amendments recite that the 2-fold increase in reticulocyte counts is seen significantly earlier than without treatment. Regarding the Examiner's concern about Figure 2, Applicant submits that Figure 2 is

a measurement of red blood cells, which are different from reticulocytes. Reticulocytes are progenitor cells that upon maturation (i.e., differentiation) become red blood cells and therefore would be expected to have a different cycle than red blood cells. Support for the claim language is found, for example, in the specification, at page 32, lines 6-8. Further support is found in Example II, at page 36, where it states that: "For sham treated animals, the reticulocyte counts went from a baseline at d=0 of 4.5% to 8.7% at d=6, and for the TPO-treated animals, the reticulocyte counts went from a baseline at d=0 of 5.3% to 12.0% at d=6." Graphical illustration of the data presented in the instant specification can be found in Grossman et al., Experimental Hematology 24:1238-1246, 1996 (copy enclosed). On page 1241, graph D, it is shown that a greater than two-fold increase in the mean reticulocyte index is seen within a 14-day period. Moreover, the sham-treated animals' recovery of reticulocytes lags significantly behind, not even beginning recovery until after day 14. This reference is supplied merely to provide additional means for evaluating the data shown in the instant application. Applicant submits that these data provide support for the claims 28-30, and that the scope of the claims are commensurate with the disclosure.

Claims 9 and 18 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. It is the Examiner's belief that claim 9 is confusing for failing to identify the acronym "TPO" and claim 18 is confusing for failing to identify the acronym "EPO".

Claims 9 and 18 have been amended respectively to recite either "thrombopoietin" instead of TPO and "erythropoietin" instead of EPO, thereby obviating the rejection. Applicant requests the rejection be withdrawn.

Claims 9-30 have been rejected under 35 U.S.C. §103 for being unpatentable over Evatt et al. and McDonald et al. It is the Examiner's belief that Evatt et al. teach the administration of partially purified human EPO and TPO

to mice, and disclose that the partially purified preparations may be capable of stimulating both platelet and red cell production. The Examiner also alleges that McDonald et al. teach a four-step procedure for the purification of TPO, which upon purification is suitable for amino acid sequencing, which in turn could lead to cloning of the gene and recombinant production of the protein. Thus, the Examiner believes that it would be *prima facie* obvious for one skilled in the art to use TPO and an erythropoietic agent to stimulate erythropoiesis.

Applicant respectfully traverses this ground for rejection. Prior to the publication of papers by Lok et al., de Sauvage et al., Kaushansky et al., and Wendling et al., in the journal Nature in June 1994, thrombopoietin was a hypothetical entity, inferred from an activity observed in thrombocytopenic plasma and certain cell-conditioned media. Pre-1994 papers which purport to show purification of what they call "thrombopoietin" (based on analogy to erythropoietin) failed to provide unambiguous identification of the factor(s) responsible for this observed activity. As summarized by de Sauvage et al., "Although these activities have been described since the 1960s, attempts to purify them have been unsuccessful." McDonald discloses that the protein, "should be useful for amino acid sequencing." Despite the alleged purity and characterization of McDonald's thrombopoietin, it was not until nine years later that a real thrombopietin, characterized by amino acid sequence, was reported. If, as the Examiner suggests in the instant Action, McDonald's sequence was sufficient "to lead to the cloning of the gene", why had no one been able to sequence the protein or clone the gene until 1994? It is noteworthy that none of the groups that reported thrombopoietin in June 1994 obtained their protein from HEK cell conditioned medium, the source of McDonald's protein.

The protein of McDonald et al. is materially different from those of Applicant. The protein of McDonald

et al. is characterized as a monomer of 15 kDa as determined by SDS-PAGE under reducing conditions, that under non-denaturing conditions self-associated to form a dimer of approximately 30 kDa. In contrast, the polypeptides recited in Applicant's claims do not dimerize under non-reducing or non-denaturing conditions and in its full-length glycosylated form, Applicant's thrombopoietin migrates at approximately 70 kDa and is predicted to migrate at about 35.5 kDa if non-glycosylated. Thus, Applicant submits that the protein disclosed by McDonald is not the thrombopoietin claimed for use as a factor for stimulating erythropoiesis in the present application.

Evatt et al. provide even less characterization of the protein(s) responsible for the putative thrombopoietic and erythropoietic activities described in the cited reference. The protein(s) isolated by Evatt et al. were obtained from fractionated thrombocytopenic rabbit plasma with the thrombopoietic activity being associated with a fraction precipitated by 60%-80% levels of saturation with ammonium sulfate. Some further fractionation was done using DEAE-cellulose and Sepahadex gel chromotography. However, Evatt et al. concede (page 552) that: "the preparations of thrombopoietin or erythropoietin were not pure, as indicated by SDS-urea gel polyacrylamide electrophoresis. Each preparation contained many bands, several of which migrated in similar locations, suggesting the possibility that similar proteins or polypeptides were contained in the preparations." Moreover, Evatt et al. do not suggest using thrombopoietin and erythropoietin in combination, but suggest that (page 548): "these findings, *in toto*, suggest the possibility that, under certain circumstances, erythropoietin may have thrombopoietic-stimulating activity." Figure 1 of the cited reference shows data for administration of either Evatt's "TPO" or EPO, not a combination, and results demonstrate that EPO is clearly superior for stimulating red blood cell production. Applicant does not find an

indication, as suggested by the Examiner (page 9 of the instant Action), that Evatt stated that the combination might be synergistic. The Examiner's position that combining something having erythropoietic activity with something having thrombopoietic activity would enhance the erythropoietic activity seems at best speculation. And while Evatt et al. suggest that protein fractions of plasma of thrombocytopenic rabbits exhibit thrombopoietic activity and some slightly stimulating effects for red cell production (Figure 1), no one skilled the art could be certain what to attribute that effect to, and certainly would not have suggested the amino acid sequence described by Applicant.

The Examiner cites *In re Kerkhoven* to support her contention that combination of two compositions, each of which is taught by the prior art to be useful for the same purpose in order to form a third composition that is to be used for the same purpose, would have been obvious to one of ordinary skill in the art at the time the invention was made. Applicant disagrees that *In re Kerkhoven* is applicable to the instant application. Kerkhoven is based on the combination of two known detergents, adequately described in the prior art. The thrombopoietin as claimed by Applicant was not identified by either Evatt et al. or McDonald et al., and therefore was not known. Applicant respectfully submits that the present invention was not obvious and requests that the rejection be withdrawn and a notice of allowability be given.

On the basis of the above amendments and remarks, Applicants believe that each rejection has been addressed and overcome. Reconsideration of the application and its allowance are requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the application, the Examiner is invited to telephone the undersigned at (206) 442-6672.

Respectfully Submitted,
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Enclosures:
Petition and Fee for Extension of Time (in duplicate)
Amendment Fee Transmittal (in duplicate)
1 Reference
Copy of Application Fee Transmittal
Copy of Application Filing Confirmation Postcard
Postcard